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Studies on the Metabolites of Phytotoxic Fungi.II. Isolation of *meso*-Erythritol, Ergosterol, Mannitol and Some Fatty Acids from *Penicillium janthinellum* BIOURGE

Russian workers isolated an antifungal antibiotic janthinellin[1] in an amorphous state from the mycerium of *Penicillium janthinellum* by extraction with methanol and precipitation with water. But the structure thereof has remained unelucidated. In an attempt to isolate this antibiotic, the present authors cultivated the same fungus (IFO strain No.4651), and extracted with methanol both at boiling and room temperatures. The crude amorphous powder deposited after addition of water showed IR absorption peaks in a fairly good agreement with those reported for janthinellin. Nevertheless, the antifungal test of this amorphous powder on *Neurospora sitophila* gave negative result. It is probable that the production of this antibiotic is highly dependent on the strain as refered to by Lisina-Kulik *et. al.*[2] In relation to this work of isolation of the antibiotic, we came across with the separation from the mycerium of various substances.

The mycerium was extracted successively with hexane, ether, and methanol for each one week in an Asahina-type extractor. The hexane extract gave colorless plates of mp 160° that had IR absorption peaks of double bond at 1640 and of hydroxyl at 3460 cm^{-1}. This substance which showed positive Liebermann-Burchard reaction of steroid and UV absorption peaks at 250(shoulder), 260(shoulder), 269, 280 and 292 *nm* was identified with ergosterol by mixed fusion and IR spectra. The methanol extract was heated with some amount of methanol and divided into soluble and insoluble fractions. The soluble fraction gave colorless microcrystalline powder, after cool, which melted at 166.5°. This substance, apparently a carbohydrate as judged by IR spectrum and Molish reaction, was derived into its acetate by the reaction with acetic anhydride in the presence of a drop of sulfuric acid. The acetate, mp 124°, thus obtained was identified with mannitol hexaacetate by mixed fusion and comparison of IR spectra. The mother liquor after separation of mannitol was evaporated *in vacuo*, and the residue was purified chromatographically on alumina with chloroform to give a thin yellow oil that crystallized when kept in an ice chest. This substance, melted at 119° after recrystallization, showed the molecular ion peak at *m/e* 122 in the mass spectrum and gave the data of elementary analysis that indicated its molecular formula $C_4H_{10}O_4$. This compound, again a presumed carbohydrate by IR and Molish reaction, was converted into its acetate by reacting with acetic anhydride and a drop of sulfuric acid. This acetate, mp 85°, was identified with the tetraacetate of authentic meso-erythritol. *Meso*-erythritol here obtained has been reportedly produced also by *Penicillium chrysogenum*[3], *Penicillium brevi-compactum*[4], *Penicillium cyclopium*[4], *Aspergillus terreus*[5], *Pichia miso*[6], *Ustilago maydis*[7], and some lichen species[8]. The methanol-insoluble part gave fatty acid mixture along with mannitol. This fatty acid mixture was treated with diazomethane and examined by gas-liquid chromatographic anaylsis. The two major fatty acids present were clarified to be palmitic and stearic by co-chromatography wih authentic specimens. The *p*-aminoazobenzene derivatives[9] of both acids gave the same melting point, 119°, and did not cause mp depression upon admixture, though well-separable from each other upon chromatography on alumina using chloroform as solvent. From 345 g of mycerium was obtained 6g, 40g, and 0.5g respectively of *meso*-erythritol, mannitol and ergosterol.

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